

Serial No. 10/622,254

RDID 02024

REMARKS

Amendment of Serial No. 10/622,254 filed July 18, 2003 is respectfully requested by Applicants. Pages showing changes made are attached hereto.

The examiner is hereby authorized to charge any fees associated with this Amendment to Deposit Account No. 02-2958. A duplicate copy of this sheet is enclosed.

Respectfully submitted,



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Data was captured on a Macintosh computer. OD₄₅₀ values were graphed for each of the various concentrations of free drug competitor (10^{-12} - 10^{-4} M).

[00126] Data from this specificity determination method was used to calculate the percent cross-reactivity of each antibody to the different drugs as compared to the immunizing drug, MDMA. This was accomplished by analyzing the data to determine the ED₅₀ for each drug. The ED₅₀ is the measure of the effective concentration of free competitor drug (MDEA, MDA, etc.) required to inhibit monoclonal antibody binding to conjugate-bound MDMA by 50%. The cross-reaction was calculated by dividing the ED₅₀ of the standard by the ED₅₀ of the drug being considered, and percent cross-reaction was calculated by multiplying the cross-reactivity by 100. This analysis showed that one clone, designated MDMA 8.3, unexpectedly showed an 89-fold higher affinity for the drug MDEA than for the immunizing drug MDMA. This clone also unexpectedly showed a 4.6-fold higher affinity for MDA than for MDMA. These findings are summarized in Table 1 below.

Table 1. Specificity determination of MDMA 8.3, % cross-reaction

Clone	MDMA	MDEA	MDA	MBDB	BDB	d-AMP	d-MAMP	l-AMP	l-MAMP
8.3	100	8,879	464	0	0	0	0	0	0

[00127] The murine hybridoma cell line MDMA 8.3 was deposited with the American Type Culture Collection (ATCC, Manassas, VA) on July 23, 2003 and assigned ATCC designation PTA 5340.

Example 46. Production of MDMA 6.1 hybridoma and monoclonal antibody

Immunizations

[00128] Female Balb/C mice 16 weeks of age or older were immunized by multiple injections of the immunogens according to the following schedule. 100 µg of MDMA immunogen 1P per mouse was mixed with an equal volume of RIBI immunogen (Sigma Chemicals) for 2-3 minutes and loaded into an appropriately sized syringe fitted with a 37 gauge hyperdermic needle. Each mouse received a 100 µg dose of immunogen with adjuvant via intraperitoneal injection. Thirty-nine days later, the same mice received

Screening

[00130] The same methods were employed as described in Example 45.

Specificity

[00131] Specificity determinations were made as described in Example 45. An antibody developed in this example, in contrast to previous findings, was unexpectedly found to show a high degree of cross-reaction for *d*-methamphetamine. This clone, designated MDMA 6.1, showed essentially the same affinity for *d*-methamphetamine and for MBDB as for MDMA, as shown in the table below.

Table 2. Specificity determination of MDMA 6.1, % cross-reaction

Clone	MDMA	MDEA	MDA	MBDB	BDB	d-AMP	d-MAMP	l-AMP	l-MAMP
6.1	100	43.1	0.3	100	3.4	0.007	95.5	0	2.1

[00132] The murine hybridoma cell line MDMA 6.1 was deposited with the American Type Culture Collection (ATCC, Manassas, VA) on July 23, 2003 and assigned ATCC designation PTA 5339.

Example 47. Production of clone and monoclonal antibody MDEA 2.2*Immunizations*

[00133] Female Balb/C mice 16 weeks of age or older were immunized by multiple injections of the immunogens according to the following schedule. 100 µg of MDEA immunogen 2U per mouse was mixed with an equal volume of RIBI immunogen (Sigma Chemicals) for 2-3 minutes and loaded into an appropriately sized syringe fitted with a 37 gauge hypodermic needle. Each mouse received a 100 µg dose of immunogen with adjuvant via intraperitoneal injection. Thirty-nine days later, the same mice received another injection identical to the first. The injections were repeated on day 60 and again on day 80. The injections were repeated on day 137, and 4 days later, one mouse was used for fusion.